Scand. J. Lab. Anim. Sci. No. 3 . 1993 . Vol. 20

Selective breeding for non-responders modifies serological response to infection with parvovirus in rats kept together with randomly bred rats

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Introduction

Susceptibility to infection and the development of disease as a result of infection is under the control of the genetics of the host (1–6). So, is the immunological response, i.e. the humoral as well as the cell-mediated response (7–11). In outbred stocks there is a much greater variation in immune response than in inbred strains (12). Genetics coding for the absence of a humoral response to certain infections are of course hazardous to health monitoring procedures. Therefore, FELASA guidelines for the health monitoring of rodents recommend that inbred strains kept in the same unit are screened successively (13).

Several rodent parvoviruses are known. The most important for rats and mice are Kilham Rat Virus (KRV) (14–16) and Toolans's H1 Virus (H1) (17) infecting rats, Minute Virus of mice (MVM) (18) infecting mice and Rodent Orphan Parvovirus (ROPV) (19–21) probably infecting both rats and mice. Parvovirus infection, especially KRV in rats and MVM in mice, are among the most common virus infections in laboratory rodents (22).

Of vital importance in the diagnostic procedures concerning these viruses is the ability to divide between the different strains, e.g. to decide whether rats are infected with KRV, H1 or ROPV. The most sensitive methods of detection are solid phase serological tests, such as enzyme-linked immunosorbent assay (ELISA) (23) or immunofluorescence assay (IFA) (24). However, all parvoviruses seem to elicit a common antigen during in vitro infection leading to cross-reactions between different types when detected by IFA and under some circumstances also by ELISA, while haemagglutination inhibition assay (HAI) is based on a specific capsid antigen (25), making this test rather specific (26). However, the sensitivity of this test is lowered by the presence of non-specific inhibitors present in many sera (27).

Productive parvovirus infection is initiated by adsorption of the virion to specific cell-surface receptors. Some differentiated cell types lack such receptors and are completely resistant to infection (27). The species-specificity making e.g. KRV a rat virus and MVM a mouse virus is probably connected to the specificity of the receptors.

We often observe that in a random sampling from rat colonies infected with parvoviruses from the same colony there is either very high titers against the virus or no titers at all, when tested by HAI. The negative results of certain sera may be due to one or several of four reasons:

a. the presence of a high amount of inhibitors in the sera
b. the absence of specific receptors in these individuals
c. lack of exposure to the virus in these individuals
d. some kind of immunodeficiency in these individuals.

If a or b should be the case, it seems reasonable to assume, that genetics are involved,

and that the ability to produce negative sera may be transferred to the off-spring.
In this study we tried to test this theory by consequently selecting seronegative animals for further breeding.

Materials and methods
Antibodies to KRV were monitored by the use of HAI, as described by *Krafi & Meier* (24), with antigen from Abtek Biological Ltd. (GB-L5 5 AD Liverpool), or Zentralinstitut für Versuchstierzucht (D-3000 Hannover). A sample with a titer less than 1:20 was regarded as negative.
A colony of Mol-WIST rats (Møllegaard Breeding Centre Ltd., DK-4623 Ll. Skensved) kept in a barrier facility was used for this study. Temperature was kept on 22° ± 2, and relative humidity was 55–80 %. The rats were fed an Altromin 1314 diet (Altromin Denmark, DK-2820 Gentofte). Prior to the study antibodies to KRV were found in 26 out of 27 sera from the colony tested by HAI. Routine health monitoring in the colony for a number of viruses, bacteria and parasites revealed the presence of *Bacillus piliformis, Staphylococcus aureus* and group B and D *Streptococci.*
A selective breeding programme was performed in the barrier unit of the original Mol:WIST colony. 25 females and 25 males were randomly sampled and tested for antibodies to KRV by HAI in the age of ten weeks. As only one seronegative female and no seronegative males were found, rats with titers ≤ 1:40 were mated one to one and from their off-spring 25 females and 25 males were randomly sampled and tested for antibodies to KRV in the age of 10 weeks. In this generation no seronegative males were found, but four males with titers ≤ 1:40 were mated with four seronegative females of the same generation. In the following five generations 25 females and 25 males per generation were sampled and tested similarly, and out of the 50 tested animals the seronegative ones were used for further breeding in the next generation. Additionally to testing by HAI all 50 sera sampled from generation 7 were tested by IFA as described by *Krafi & Meier* (24) with antigen from Zentralinstitut für Versuchstierzucht (D-3000 Hannover) and by ELISA as described by *Smith & Gehle* (28) with antigen from Organon Teknika Corp. (USA-27704-0969 Durham). Titers above 1:20 in IFA were considered positive, while ELISA-results were interpreted on the basis of the optical density-values as described by *Andersen et al.* (29).
During the 97 weeks of selective breeding eight rats above the age of ten weeks were sampled every three months from the original Mol:WIST colony kept in the same unit. Sera from these rats were also tested for antibodies to KRV in HAI.

Results
The results are shown in figure 1. After seven generations of selective breeding the prevalence observed by HAI testing was reduced from 100 % to 0 %. In generation 7 all 50 rats were also negative when tested by ELISA. However, by the use of IFA 24 out of 25 males and 19 out of 25 females were positive.
Screening of sera by HAI from the original Mol:WIST colony in the same period resulted in 39 positives out of 56, the prevalence ranging from 63 % (5/8) to 100 % (8/8).

Discussion
The prevalence of seropositives to KRV in HAI was evidently reduced by selective breeding. As the original colony kept in the same unit remained seropositive through the whole observation period lack of exposure to the virus is not a very likely explanation for the negative results. It seems reasonable to assume that the reduced prevalence from generation to generation was due to a genetic trait. Whether the selection was based on the lack of specific receptors or a higher production of inhibitors in the sera of the selected individuals or maybe on a combination of the two cannot be fully concluded by this

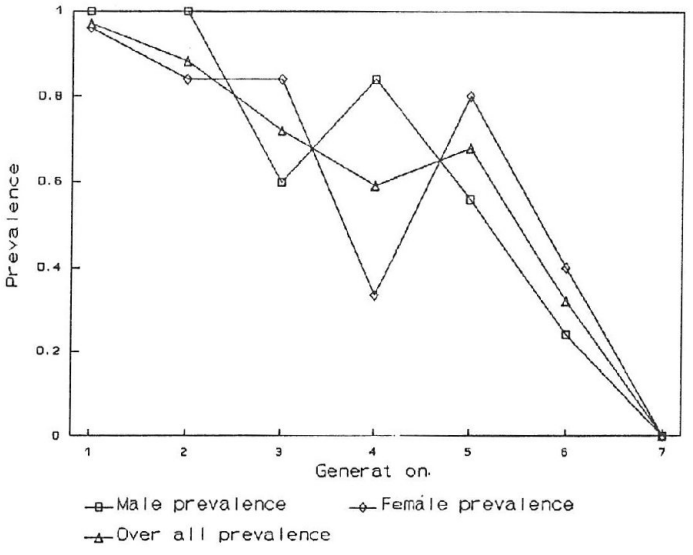

Figure 1. Prevalence of antibodies to Kilham Rat Virus (KRV) in a colony of Mol:WIST laboratory rats selected for breeding on the basis of absence of antibodies to KRV in haemagglutination inhibition assay.

study, as no experimental exposure to KRV was performed. However, the rats of generation 7 also being negative by ELISA test while positive by IFA may be explained by the rats being infected with two parvoviruses, one of these not being eliminated by selection based on the specific HAI-test. The ELISA-test used in this study cross-reacts with H1, but it may not cross-react with ROPV. So, if the rats were simultaneous infected with ROPV, this may explain why the sera came out positive when tested by IFA.

The results may be explained by the lack of immunological capability developed through the generations. If this were the case it should be expected that the non-responders would be more susceptible to the infection and its consequences. However, as the rats of generation 7 made antibodies against KRV in IFA a general immunodeficiency does not seem to be a reasonable explanation for the absence of positive results in HAI.

Further studies are needed to explain these results. If rats lacking specific receptors could be produced by this method it may be used for preventing parvovirus infection and its influence on research in rodent colonies, as far as characteristics essential for the animal model are not eliminated.

So far, our study has shown that it is wise to consider genetics when doing routine health monitoring, e.g. by successive monitoring of inbred strains kept in the same unit as proposed by the FELASA working group on health monitoring (13).

Summary

In a colony of Wistar rats antibodies to Kilham Rat Virus in haemagglutination inhibition assay were found in the sera of 26 out of 27 animals sampled. While the prevalence in the original colony still maintained in the same unit did not change significantly in the same period, selection of breeding animals based on the absence of such antibodies over seven generations reduced the prevalence in the selected colony to 0 %.

Sammendrag
I en koloni af Wistar-rotter blev der i haemagglutination inhibition assay påvist antistoffer mod Kilham Rat Virus i serum fra 26 ud af 27 dyr. Mens prævalensen i den oprindelige koloni, der stadig blev avlet i den samme dyrestald, ikke ændredc sig væsentligt i den samme periode, medførte selektion af avlsdyr baseret på fravær af disse antistoffer en reduktion af prævalensen til 0 % i løbet af 7 generationer.

Yhteenveto / K. Pelkonen
Eräässä rottakoloniassa löydettiin hemagglutinaationhibitiomenetelmällä vastaaineita Kilham Rat virukselle 26 seerumissa 27 tutkitusta. Kun siitokseen valittiin seitsemän sukupolven ajan vain eläimia, joilla ei ollut vasta-aineita, päästiin valinnalla lopulta puhtaaseen koloniaan, jossa vasta-aineita löytyi 0 %:ssa tutkituista, vaikka alkuperäistä koloniaa samanaikaisesti edelleen tuotettiin samassa yksikössä, eikä siinä vasta-aineiden yleisyyys merkittävästi muuttunut.

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